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Ox meat traceability: Practical implementation using electronic identification and molecular markers

J. Egozcue*, P. Lavin, B. Soret* and A. Arana***
*Instituto de Agrobiotecnología (UPNA-CSIC), Carretera Mutilva Baja s/n
31192 Mutilva Baja (Navarra), Spain
**Estación Agrícola Experimental (CSIC), Finca Marzanas, 24346 Grulleros (León), Spain

SUMMARY **–** An integral control system to assure meat traceability from producers to consumers was developed. After birth, oxen are identified by a ruminal bolus electronic device (transponder) placed in the animal forestomach until slaughter. The registered transponder number identifies the animals during their whole life and after slaughter, it also identifies the carcasses and the commercial retail cuts. To guarantee meat traceability throughout the whole meat chain, a DNA-based control and verification procedure was established and its implementation evaluated. In this work the actions performed to establish the procedure to audit the traceability system are described. In order to create a bank of ox samples, an individual identification card containing the identification data and a blood sample on filter paper was elaborated. Once prepared, the card was laminated and stored at room temperature. The implementation of the control procedure consisted of a comparison between the DNA marker profiles of the meat samples collected anonymously at the butcher's or restaurant, and the DNA markers profiles of the blood on the identification card. This procedure was successfully completed.

Keywords: Traceability, electronic identification, DNA markers, ox.

RESUMÉ – *"Traçabilité de la viande de bœuf : Mise en œuvre pratique en utilisant l'identification électronique et les marqueurs moléculaires" Pour assurer la traçabilité de la viande de bœuf, on a développé un système intégral de contrôle, qui depuis le secteur primaire jusqu'à la transformation, permet de garantir la viande qui arrive aux consommateurs. À cet effet, l'animal est identifié après la naissance avec un dispositif électronique (ruminal boluses) qui reste dans le rumen-réticulum jusqu'à ce qu'il soit abattu. Le numéro de registre du dispositif est celui qui identifiera l'animal vivant, sa carcasse et les pièces. Pour contrôler le système de traçabilité, une procédure de contrôle basée sur l'identification génétique des animaux (analyse de marqueurs d'ADN) a été établie et sa mise en œuvre a été évaluée. Dans ce travail, les essais effectués pour établir ce système sont présentés. Dans le but de disposer d'une banque d'échantillons des animaux, on a élaboré une carte identificative individuelle en papier de filtre qui contient un échantillon de sang. Cette carte sera plastifiée et conservée à température ambiante. On a évalué la mise en œuvre de la procédure de contrôle du système de traçabilité en comparant des profils de marqueurs d'ADN pour les échantillons de viande rassemblés, d'une manière anonyme, chez le boucher ou le restaurant et les échantillons de sang conservés dans les cartes d'identification. Cette évaluation a été très satisfaisante.*

Mots-clés : *Traçabilité, identification électronique, marqueurs d'ADN, bœuf.*

Introduction

Nowadays, consumer demands for meat products from quality assurance schemes is increasing. For this schemes, traceability is one of the most important tools as it assures the relationship between the animal and the meat cuts through the whole chain of production, transformation, distribution and commercialisation.

In the last years, meat commercialised as certified quality brands, Geographic Protected Identification (GPI), etc., has increased. These brands offer a high quality product very appreciated by the consumer and a guarantee of its origin and production techniques.

The Quality Brand *"Valles del Esla"* was created ten years ago with the goal of producing high quality and guaranteed traceability cattle meat. One of the products of this brand is the ox meat, obtained from Brown Swisse castrated males aged more than four years old. These animals are reared on pasture in the mountains of León (Spain) and fed indoors for a small period of time obtaining a high value product .

For assuring meat traceability from producers to consumers the brand developed an integral control system. After birth, animals are identified by a ruminal bolus electronic device (transponder) placed in the forestomach of the animal until slaughter (Caja *et al*., 1999). The registered transponder number identifies the animals during its whole life and the carcasses and cuts after slaughter.

To guarantee meat traceability and detect possible failures, a control and verification procedure through the whole chain needs to be established. The application of genetic (DNA markers) profiling methods to assure animal and meat traceability has revealed as a useful tool to trace any piece of meat from slaughter to consumer and to link it with the animal identity (Cunningham *et al*., 1999; San-Cristóbal *et al*., 2000; Arana *et al*., 2002). Traceability genetic methods are based on the comparison of genetic information (DNA markers) of the animal biological samples (blood, hair, etc.) with the meat of that animal. To verify "a posteriori" the origin of the meat it is necessary to elaborate a bank of samples for all the animals reared by the brand.

In this work the actions performed to establish the control procedure of the traceability system are presented. The control system will be based in the comparison of the DNA markers profiles between the meat samples and samples of the animals kept in individual identification cards stored in a bank of samples for a period longer than 4 years.

The objectives of the present work were: (i) to choose the biological sample to be analysed and the sample support; (ii) to evaluate the effects of different conditions on the DNA sample conservation; and (iii) to establish a traceability control system based in the comparison of the DNA molecular markers.

Material and methods

DNA source

DNA from samples of blood, hair and auricular cartilage were extracted and analysed.

Sample support type

Blood samples from 5 different animals were analysed. Three different types of biological sample supports were embeded with blood from the 5 animals:

- Paper A: special filter paper for collection, transport and storage at room temperature during long periods of time.
- Paper B (without treatment): filter paper of 0.5 mm thickness and 250 grs/m^2
- Paper C (with treatment): filter paper similar to B treated with antibiotic [5 µl of cloranfenicol solution (3.2 mg/ml)].

The paper was included in the laminated identification card. The identification card is a 125x75 mm laminated cardboard card specifying the information of the animal and the filter paper including the DNA biological sample (Fig. 1).

Handling of the samples

Blood samples were collected and transported to the laboratory in less than 5 h. In the laboratory 100 µl of blood of each animal were transferred to the filter paper and dried two hours at 80ºC.

Long period sample conservation

Blood samples in the laminated identified card, were treated, separately or simultaneously during seven days with high temperature (60ºC) and saturated humidity in order to simulated conditions that can affect blood conservation and card lamination. Analyses were carried out in triplicate.

Fig. 1. Animal identification card.

DNA extraction and genetic profile analysis

A small piece (3 mm^2) of filter paper containing the blood sample was introduced in an eppendorff tube. Blood DNA was extracted using a commercial kit following manufacturer recommendations. PCR amplification of 11 molecular markers: BM 1824 and 2113, TGLA 53,122,126 and 227, ETH 3,10, 225 and INRA 23 and SPS 115, were performed using *StockMarks for Cattle® Bovine Genotyping Kit* (Applied Biosystmes). Molecular markers were analyser in a capillary automatic sequencer (ABIPRISM 310, Applied Biosystems) and resulting profiles interpreted by GeneScan Software (Applied Biosystems). Animal genotyping was performed with Genotyper software.

Traceability system control and verification

In order to control the traceability system and verify that the genetic identification can be implemented as an audit tool, a bank of ox blood samples was created. This are the "reference samples". Blood samples were incorporated to the animal identification card and was laminated.

In order to have the "verification samples", there were selected six different points of the commercialisation chain (at butcher's or restaurant's). At these points, meat samples were taken out and stored in a freezer (-20ºC) until required for analysis. Meat samples were collected in an anonymous way by the brand staff. According to the Sales Department the selected meat samples correspond to 26 oxen identified by its transponder number, then this 26 identifications cards were taken out of the sample bank for analysis of the genetic profile.

Samples (fresh and cooked meat and blood from the identifications cards) were analysed according to the methodology above described. The allelic profiles were compared and relatedness coefficients calculated as indicated by Arana *et al*. (2002).

Results and discussion

Initially, DNA from samples of blood, hair and auricular cartilage were extracted and analysed. Hair samples were rejected to avoid failures and mixtures on sampling. Blood samples are more adequate than cartilage samples because collection of blood samples allows to extend the period of sampling since the cartilage has to be collected when the ear-tag is placed in the animal in order to assure the animal welfare and maximize the efficiency of the process. To avoid contamination of the samples, blood is extracted using a sterile syringe and stored in a vacuum heparinized tube.

Referring to the election of the sample support (different filter papers) for DNA extraction and genetic analysis, DNA molecular markers were amplified and analysed from the three different filter papers tested (Fig. 2).

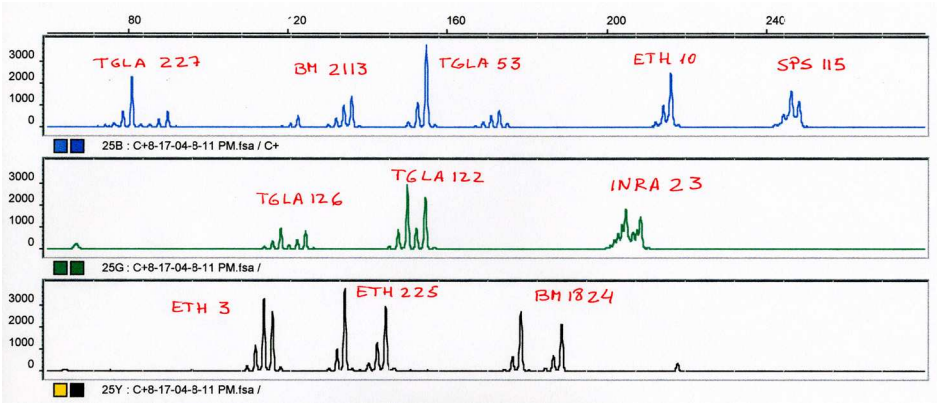

Fig. 2. Eleven DNA microsatellites analysed by Genescan software (Applied Biosystem).

The DNA preservation is an important aspect to be guaranteed for the genetic traceability control system since ox production lasts more than 4 years. Conditions of temperature and saturated humidity during a week did not affect molecular markers analysis. DNA profiles were obtained in these conditions from the three different kinds of supporting papers.

When elaborating a traceability control system the economic aspect is essential. In the proposed traceability system the cost of the identification card, including the filter paper for blood sample, has to be taken into account since all the animals have to be registered with an individual card.

Given that there were not differences between filter papers as DNA conservation is concerned, the election of the support to be used depends on its price. Table 1 shows the cost of an identification card depending on the price of the filter paper utilized to embed the blood sample and the material of the identification card. Identification card A is made in a usual paper card and the filter paper for storing the blood sample is the special filter *Paper A*. Card type B is made in usual paper card and contains the more economic filter *Paper B* to embed the blood. Identification card C is all made in filter *Paper B* and blood is poured directly to a specific place in the card. This last card C was chosen because all the data of the animal can be printed in the card directly from a computer avoiding possible failures in its translation.

Table 1. Economic cost of different identification cards

	Card A (euros/card)	Card B (euros/card)	Card C (euros/card)
Cardboard card	0.0151	0.0151	
Filter paper B card			0.03
Laminated	0.148	0.148	0.148
Sample support: Paper A	1.1125		
Paper B		0.0022	
Total	1.2756	0.1653	0.178

Besides the cost of the identification cards, other costs should be taken into account as related to sample collection process and genetic analysis. The former is difficult to be evaluated since the collection of blood samples is carried out at the same time than other tasks as animal data collection, feed sampling or transponder insertion. Although the cost of the molecular analysis is high (more than 30 euros/sample) it can be assumed because it is an audit tool and only a small number of samples, corresponding to a small percentage of animals, is analysed.

To store all the data of the animals reared and commercialized under this quality brand, a computer database was designed. The identification card is printed directly, from the database

minimizing failures on data transcription. The transponder number of the animal, its administrative ear-tag number, birth date, farm number, animal type and breed, date of blood collection and number of the blood tube were printed in the identification card.

In relation to the implementation of the traceability control and verification system proposed by the brand, the DNA from 6 meat samples collected at the butcher's or at the restaurant and also the DNA of 26 identification cards corresponding to the animals related to the meat samples was extracted and analysed. The genetic profiles of the meat samples were compared with the DNA profile coming from the cards. In all the cases, after computing relatedness coefficients between samples it was verified that every meat sample agrees with one of samples extracted from the identification cards. These results indicates that the implementation of the DNA traceability control procedure was successful and that there is an unique correspondence between the meat and the ox of origin, as guaranteed by this quality brand.

Conclusions

In summary, DNA marker methodology has been shown to be an appropriate approach to establish the origin of a beef cut. DNA based traceability is simple and can be useful for meat to increase consumer's trust and add value to the meat. Its application to the market conditions means that its implementation should have an affordable cost that could be assumed by high quality products.

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References

Arana, A., Soret, B., Lasa, I. and Alfonso, L. (2002). Meat Traceability using DNA markers: Application to the beef industry. *Meat Science,* 61: 367-373.

Caja, G., Conill, C., Nehring, R. and Ribó, O. (1999). Development of a ceramic bolus for the permanent electronic identification of sheep, goat and cattle. *Computers and Electronics in Agriculture*, 24: 45-63.

Cunningham, E.P., Meghen, C., Bradley, D.G., MacHugh, D.E. and Loftus, R.T. (1999). DNA Traceability techniques for the meat industry. In: *New developments in guaranteeing the optimal sensory quality of meat*, Toldra, F. and Troy, D.J. (eds), Valencia (Spain), 5-7 May 1999, pp. 1-7.

San Cristobal-Gaudy, M., Renard, G., Amigues, Y., Boscher, M.Y., Leveziel, H. and Bibe, B. (2000). Traçabilité individuelle des viands bovines a l´aide de marqueurs génétiques. *INRA Prod. Anim.*, 13(4): 269-279.